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JOURNAL ARTICLE

Comparative Toxicology of Laboratory Organisms for Assessing Hazardous Waste Sites[1]

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ABSTRACT

Multi-media/multi-trophic level bioassays have been proposed to determine the extent and severity of environmental contamination at hazardous waste sites. Comparative toxicological profiles for algae (*Selenastrum capricornutum*), daphnia (*Daphnia magna*), earthworms (*Eisenia foetida*), microbes (*Photobacterium fisherii*, mixed sewage microorganisms) and plants; wheat "Stephens," (*Triticum aestivum*), lettuce, butter crunch, (*Lactuca sativa* L.), radish, "Cherry Belle," (*Raphanus sativa* L.), red clover, "Kenland," (*Trifolium pratense* L.) and cucumber, "Spartan Valor," (*Cucumis sativa* L.) are presented for selected heavy metals, herbicides and insecticides. Specific chemical EC_{50} values are presented for each test organism. Differences in standard deviations were compared between each individual test organism, as well as for the chemical subgroup assayed. Algae and daphnia are the most sensitive test organisms to heavy metals and insecticides followed in order of decreasing sensitivity by Microtox (*Photobacterium fisherii*), DO depletion rate, seed germination and earthworms. Higher plants were most sensitive to 2,4-D, (2,4-Dichlorophenoxy acetic acid) followed by algae, Microtox, daphnia and earthworms. Differences in toxicity of 2,4-D chemical formulations and commercial sources of insecticides were observed with algae and daphnia tests.

Additional Index Words: **comparative toxicology, heavy metals, hazardous waste, complex chemical mixtures.**

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Dowd (1984), in an editorial on biological monitoring discusses the rationale for a trend toward increased use of biological measurements to define the health of an organism or ecosystem. He attributes this trend to an increased awareness that trace chemicals in our environment may contribute in a larger way than previously expected, to the integrated ecotoxicological effect of environmental components. Inability to interpret the toxic potential of trace amounts of numerous chemicals has stimulated interest in the direct use of biological assays to help define the integrated effects of complex chemical mixtures upon human health and environmental systems. Dowd suggests that in some cases data obtained by biological monitoring may eliminate the need for chemical measurement. While this may be an over simplification, the concept of using bioassays to support and/or direct comprehensive chemical characterization of environmental samples has been presented by Miller et al. (1978). The major advantage of this approach is that the biological response to a complex mixture of chemicals integrates the effects of environmental variables such as solubility, pH, antagonism, synergism, and time of exposure, all of which affect toxicity to the test organisms. Chemical priority pollutant analysis of industrial effluents, hazardous waste site soil, surface water and ground water samples is usually expensive and may not insure the attainment of useful environmental risk assessment data. Samoiloff et al. (1983) found that the most toxic fractions in lake sediments contained none of the EPA consent decree priority pollutants. Other criticisms of use of chemical water and air quality criteria values to predict toxicity are that: (i) Criteria applications tend to be ultra conservative and thus overly restrictive. (ii) Data bases are inadequate to establish criteria for most chemicals. (iii) The available chemical criteria are usually not the ones needed at complex waste disposal sites. (iv) There is little evidence to indicate that the effects of individual chemicals is additive under field conditions. (v) And, for contaminated soils, there are no criteria available to make any judgments concerning clean-up needs.

These concerns caused the Hazardous Materials Assessment Team (HMAT) to focus on the development, refinement and validation of a laboratory bioassessment protocol (Porcella, 1983) for defining the degree of environmental toxicity potential associated with hazardous waste sites. This paper discusses the comparative toxicology of the HMAT test protocol organisms to representative components of three chemical subgroups (heavy metals, insecticides, and herbicides)

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Table 1. Test organism mean EC_{50} values, standard deviation, and coefficient of variation for heavy metal, herbicide, and insecticide chemical subgroups.

Test organism	Heavy metals				Herbicides				Insecticides			
	EC_{50}	SD	cv	N†	EC_{50}	SD	cv	N	EC_{50}	SD	cv	N
	mg/L				mg/L				mg/L			
Algae	0.042	0.005	12	12	54.35	19.02	35	8	0.127	0.019	15	20
Daphnia	0.22	0.04	20	12	>126.55	41.76	33	8	0.138	0.026	20	20
DO Rate, 3 h	0.50	0.88	35	6		-				-		
Microtox, 30 min	4.98	1.24	25	20	68.20	9.82	14	8		NE‡		
Plant seeds	67.93	13.59	20	15	<0.17	0.017	10	20		NE		
Earthworm	623.90	124.80	20	9	11 315	2 772	25	8	88.33	23.84	27	15

† Standard deviation, mean EC_{50}, coefficient of variation [(standard deviation/mean) × 100] and number of tests respectively.
‡ NE = No significant toxicity was observed.

Table 2. EC_{50} response to Cu, Cd and Zn.

Test organism	Copper	Literature values	Cadmium	Literature values	Zinc	Literature values
	mg/L					
Algae	0.042	0.034†	0.041	0.050‡	0.066	0.100‡
Daphnia	0.064	0.030§	0.041	0.047§	0.54	0.66¶
DO 0 h	27.2	-	-	-	5.1	-
3 h	3.5	5.0#	-	-	1.5	-
22 h	3.0	-	-	-	39.7	-
Earthworm (H_2O)††	168	376††	997	1440‡‡	707	960‡‡
Earthworm (Soil)††	644	-	1834	-	628	-
Microtox 5 min	1.2	1.8§§	106	154§§	12	56.0§§
15 min	0.42	0.28§§	25	41§§	1.6	6.1§§
30 min	0.24	0.13§§	14	15§§	0.7	1.4§§
Wheat	24	-	66	76¶¶	334	-
Lettuce	22	-	58	61¶¶	31	-
Radish	55	-	58	128¶¶	53	-
Red Clover	45	-	57	27¶¶	43	-
Cucumber	47	-	65	102¶¶	61	-

† Chiaudani and Vighi, 1978.
‡ Bartlett et al., 1974.
§ Canton and Adema, 1978.
¶ U.S. EPA, 1980a.
# Bauer et al., 1981.
†† LC_{50} values = concentration at which 50% mortality occurs.
‡‡ Neuhauser, personal communication, 1983.
§§ Qureshi, A. A. et al., 1983.
¶¶ Ratsch, 1983.

as a basis for understanding their response to complex waste mixtures (metals and organics) in soil, soil elutriate, and pond water samples from the Western Processing Waste Site near Kent, WA. Toxicity values are interpreted as high (< 20% elutriate), moderate (20–75% elutriate), and low (75–100% elutriate) according to Porcella (1983).

METHODS

All chemicals, waste site soils, soil elutriates, and surface water samples were processed and assayed with algae (*Selenastrum capricornutum*), daphnia (*Daphnia magna*), earthworm (*Eisenia foetida*), wheat (*Triticum aestivum*), lettuce (*Lactuca sativa* L.), red clover (*Trifolium pratense* L.), radish (*Raphanus sativa* L.), and cucumber (*Cucumis sativa* L.) seed germination tests, according to Porcella (1983). The Microtox (*Photobacterium fisherii*) tests were conducted according to (Beckman, 1982). The dissolved O_2 depletion rate tests (mixed sewage microorganisms) were conducted according to the method of Bauer et al. (1981). Heavy metals (Cu, Cd, and Zn) were prepared from ACS reagent grade chloride salts dissolved in deionized water. Water soluble fractions of Aldrin (1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonapthalene), Dieldrin (1,2,3,4,10,10-Hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonapthalene), and Endrin (1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,endo-5,8-dimethanonapthalene) were prepared from reagents supplied by Chem Service, Inc., Westchester, PA and the EPA Repository for Toxic and Hazardous Materials, Cincinnati, OH. The purity for Aldrin (6-152), Dieldrin (6-115a) and Endrin (5-171) supplied by Chem Service was 99%. The reference analytical standards supplied by the EPA repository for Aldrin, Dieldrin, Endrin, Chlordane (1,2,4,5,6,7,8,8 - Octachloro - 2,3,3a,4,7,7a - hexahydro - 4,7-methanoindene), and Heptachlor (1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) were certified to be > 97% pure and contained 5000 ± 250 mg/L in methanol. The 2,4-D (2,4-Dichlorophenoxy acetic acid) was supplied by Dow and was 94% pure. The Dow Esteron 99 (propylene glycol butylether ester of 2,4-D) contained 2,4-D at a concentration of 485 mg/L.

The chemicals supplied by the U.S. Environmental Protection Agency (EPA) Repository were added to deionized water at their water solubilities identified in U.S. EPA *Water-Related Environmental Fate of 129 Priority Pollutants* (1979). The Chem Service supplied aldrin, dieldrin and endrin were added to deionized water at 10 times their water solubility mixed with a magnetic spinbar for 48 h at 24 ± 2°C, and filtered through a 0.45 μm membrane filter prior to bioassay and chemical analysis. Dow Esteron 99 and 2,4-D were assayed at nominal concentrations ascribed to their water solubility (Worthing, 1979). Esteron was assayed as an emulsion in deionized water, while 2,4-D was dissolved in alkaline (pH > 100.00 with NaOH) deionized water which was neutralized to pH 7.0 with HCl prior to bioassessment. Concentrations of Aldrin, Dieldrin, Endrin, 2,4-D and Esteron 99 in excess of water solubility were prepared by using Spectro grade acetone prior to assay with the earthworm and Microtox test. Solvent blanks were also assayed to determine their toxicity to the test organisms.

RESULTS AND DISCUSSION

Response of Bioassays to Chemical Subgroups

The EC_{50} response values (LC_{50} for earthworms) for each of the chemicals investigated were calculated by linear regression analysis of dose/response data. Specif-

ic EC_{50} chemical concentrations are reported as average values obtained from replicate tests conducted with each test organism. The average EC_{50} values and their respective standard deviations for heavy metals (Cu, Cd, Zn), herbicides (2,4-D, Esteron 99), and insecticides (aldrin, dieldrin, endrin, chlordane, heptachlor) are shown in Table 1. Differences in standard deviations were compared between each individual test organism, as well as for the chemical subgroups assayed. Algae EC_{50} coefficient of variation values for each of the three chemical subgroups ranged between 12 and 35%. The dissolved oxygen depletion rate test had the highest coefficient of variance in the metals subgroup. The other microbial test (Microtox) had a variation of 25%. The Microtox test coefficient of variation in the herbicide subgroup improved to 14%.

Microtox EC_{50} values could not be obtained for the maximum water soluble concentration of insecticides. The plant seed coefficient of variance improved from 20% in the heavy metals subgroup to < 10% in the herbicide group. Earthworm LC_{50} coefficients of variance were 20% for heavy metals, 25% for herbicides and 27% for insecticides. The response values are discussed below by chemical groups, i.e. heavy metals, herbicides and insecticides.

Heavy Metals

Each of the tests (with the exception of DO depletion rate) identified an EC_{50} value for Cu, Cd, and Zn. The literature EC_{50} and LC_{50} values shown in Table 2 are in good agreement with those obtained in this study, with most values differing less than one order of magnitude. The values cited for earthworms in Table 2 were from (E. F. Neuhauser, 1983, personal communication). An evaluation of the heavy metal response data in Table 2 shows algae and daphnia to be the most sensitive to copper, cadmium and zinc followed in order of decreasing sensitivity by Microtox, DO depletion rate, seed germination and earthworms.

Herbicides

Two herbicides, 2,4-D acid, and esteron 99 were tested. Except for wheat, EC_{50} responses were obtained for all seed germination/root elongation test species at 2,4-D concentrations of < 0.1 mg/L (Table 3). Algae on the other hand were about 500 times less sensitive to 2,4-D. This is most likely due to the fact that 2,4-D is an auxin analog, which stimulates destructive growth in higher plants that respond to auxin growth regulation. Algae do not have auxin growth regulators, but react to inhibition of the photosynthesis energy transport process. Thus, the effects of 2,4-D on higher plants and algae differ markedly.

Differences in sensitivity levels of the test organisms relative to the toxicant assayed can be used to identify those trophic level components most susceptable to the presence of a toxicant and to make an educated estimate of the contaminant type producing the toxic effect. In this instance 2,4-D severely impacted seeds while algae, Microtox and daphnia tests were far less sensitive to similar levels. Earthworms tolerated the highest 2,4-D concentrations as was the case with metals. This rank order of sensitivity is dramatically different from that observed with heavy metals where seeds had the second lowest sensitivity. Development of a more complete bioassay data base of this nature should strengthen our confidence in bioassays to identify toxicity potential and to help focus chemical analysis where toxicity is deemed a problem.

Table 3. EC_{50} Response to 2,4-D and Dow Esteron 99.

Test organism	2,4-D	Esteron 99
	mg/L	
Algae	95.8	12.9
Daphnia	>240	13.1
Earthworm (H_2O)	NE†	NE
Earthworm (Acetone)‡	9 830	12 800
Microtox 5 min	112	10.3
15 min	107	10.3
30 min	128	8.4
Wheat	0.7	0.2
Lettuce	<0.1	<0.1
Radish	<0.1	<0.1
Red clover	<0.1	<0.1
Cucumber	<0.1	<0.1

† NE = No significant toxicity was observed.
‡ Reported as LC_{50} values.

The response of the test organisms (Table 3) to Esteron 99, a commercial formulation of 2,4-D with undisclosed "inert" constituents, shows Esteron 99 to be significantly more toxic to algae, daphnia, and Microtox than 94% purity 2,4-D acid. The response of the five plant seeds was similar for both Esteron 99 and 2,4-D. The daphnia and algae sensitivity levels are similar for Esteron 99 but are 18 and 7.4 times more sensitive to Esteron 99 than to 2,4-D acid respectively, when comparing their EC_{50} concentrations listed in Table 3. This increased daphnia and algae toxicity to Esteron 99 suggests the "inert" components in the formulation might be the active toxicants.

These results tend to reinforce the assertion of Samoiloff et al. (1983) and Dowd (1984) that bioassays can define the bioactive components in complex mixtures where chemical components are not well known or defined toxicologically. It also indicates some of the potential problems of "predicting" the toxic effect of complex chemical mixtures from pure compound toxicity test results when commercial formulations may differ significantly. For example, the Esteron 2,4-D formulation used in these tests would have been predicted to have minimal effects on aquatic organisms based on its 2,4-D content. However, bioassays of the complex mixture (as it would be used commercially) indicate the material can be quite toxic.

Insecticides

Water soluble solutions of aldrin, dieldrin and endrin prepared from reagents supplied by Chem Service and the EPA repository of toxic and hazardous materials were assayed to determine whether toxic differences in > 97% purity chemical formulations of the same generic compound can be identified with the test organisms recommended in the HMAT protocol. The EC_{50} responses shown in Table 4 indicate that algae and daphnia are the only test organisms that responded to both of the water soluble formulations of aldrin, dieldrin and endrin. Chemical analysis of these compounds is shown in Table 5.

Table 4. EC_{50} Response to water soluble concentrations of aldrin, dieldrin, endrin, chlordane, and heptachlor.

Test organism	Aldrin CSI‡‡/ EPA¶¶	Literature value	Dieldrin CSI‡‡/ EPA¶¶	Literature value	Endrin CSI‡‡/ EPA¶¶	Literature value	Chlordane EPA	Literature value	Heptachlor EPA	Literature value
	mg/L									
Algae	0.025/0.100	–	0.055/0.071	–	0.048/0.114	–	0.156	–	0.194	0.040†
Daphnia	0.025/0.094	0.028‡	0.079/0.107	0.190	0.051/0.112	0.352§	0.165	0.058¶	0.162	0.070#
DO-O h	–/0.079	–	NE/NE††	–	NE/NE	–	0.113	–	0.129	–

† U.S. EPA, 1980b.
‡ Johnson and Finley, 1980.
§ U.S. EPA, 1980c.
¶ Randall, 1979.
# Macek et al., 1976.
†† NE = No significant toxicity was observed.
‡‡ Chem Service, Inc., Westchester, PA.
¶¶ EPA Repository for Toxic and Hazardous Materials, Cincinnati, OH.

The algae and daphnia EC_{50} values for the aldrin, dieldrin, and endrin samples from the EPA repository (Table 4) are much higher (less toxic) than the EC_{50} values for the insecticides supplied by Chem Service. Methanol added to solutions (50 μL/L) of algal assay medium and daphnia test water (200 mg/L hardness) did not adversely affect either test organism at maximum methanol solvent concentrations assayed in the EPA repository insecticide samples. The higher toxicity obtained in the Chem Service insecticides suggests the presence of a toxic constituent not present or bioactive in the EPA chemical reference standards. These results further demonstrate the ability of bioassays to define chemical formulation toxicity differences among chemicals of the same generic classification thereby building our confidence that response differences to complex waste environmental samples are indeed an indication of discrete differences in toxicity and not simply noise level responses.

The response of the HMAT test organisms to the three chemical subgroups used in this study demonstrate the ability of bioassays to define the bioactive components in complex chemical formulations where the inert chemical components are not known nor defined toxicologically. Changes in tolerance levels of the HMAT test organisms were also observed relative to the toxicant assayed. These results support the position that a suite of test organisms should be used to identify those ecosystem components most susceptable to the exposure of waste types whose specific chemical and toxicity characteristics are not well known or understood.

These assay results indicate that algae may be the most sensitive aquatic test organism and that they most likely will respond to water soluble major chemical subgroups found in many hazardous waste site soils. These results also suggest that a more sensitive soil test should be chosen and evaluated in order to compliment the earthworm test.

Environmental Waste Site Toxicity Assessment

A study was initiated on soils, soil elutriates, and surface water samples from the Western Processing site, Kent, WA. The purpose was to ascertain the response of the HMAT test organisms to various mixtures of chemical subgroups and to determine whether their response could have been predicted based on extrapolation of priority pollutant chemical concentrations to biotic effects (LD_{50}).

The priority pollutant organic content of the four Western Processing soil elutriate and pond water bioassay samples is presented in Table 6. With the exception of the phenol and nitrosamine contained in sample #017, no other priority organic chemicals exceeded the EPA fresh water and drinking water criteria value (Sittig, 1980). The U.S. EPA maximum allowable level for phenol in both fresh water and drinking water is 3.4 mg/L. Fresh water criteria have not been established for nitrosamines but no nitrosamine content is allowable in drinking water. The phenol content in sample #017 is approximately six fold greater than the allowable criteria value, suggesting that this sample should be highly toxic to the aquatic test organisms. The bioassay results (Table 7) indicate this sample to be non toxic for daphnia, moderately toxic to Microtox and on the border line of being highly toxic to algae. The EC_{50} values reported in the U.S. EPA *Phenol Ambient Water Quality Criteria* document (1980d) are 40, 36, and 36 mg/L, respectively for algae, daphnia, and fathead minnow. These reported values suggest: (i) that the EPA phenol freshwater criteria value is conservative and (ii) that the toxicity observed in sample #017 does not correlate with its phenol content. The inability to predict the toxicity potential of the five Western Processing samples on the basis of their priority organic chemical content and the predominant imbalance between their total and priority organic content (Table 6) casts doubt on the use of organic priority pollutants to predict toxicity potential of complex waste samples. The imbalance observed between priority and nonpriority organic content also causes analytical problems. Neither sample concentration nor dilution can be used to increase the sensitivity of priority pollutant detection in samples which contain high background levels of organics. This high organic background may be reduced by selective chromatography separation, but the methods are time consuming, expensive and depending on the separation required, are in the developmental stage. Even if a more vigorous analysis of the priority organics in these five Western Processing samples had been conducted, it would not have predicted toxicity potential because this effort would also have missed the potentially toxic nonpriority organics. The inability to ascribe the toxicity response shown in Table 7 to priority organic

Table 5. Concentrations of aldrin, dieldrin, endrin, chlordane, and heptachlor contained in water soluble assay samples.

Constituent	Source: Chem Service	Source: U.S. EPA Repository
	mg/L	
Aldrin	0.025	0.125
Dieldrin	0.096	0.130
Endrin	0.069	0.142
Chlordane	–	0.206
Heptachlor	–	0.197

Table 6. Concentration of priority and nonpriority organics contained in western processing samples.

Constituent	East ditch control	Pone water	No. 005	No. 017	No. 020
			mg/L		
Phthalates	0.002	0.010	0.001	0.011	0.002
Ethylbenzene	0.002	<0.001	0.001	0.001	0.001
Nitrosamines	<0.001	<0.001	<0.001	0.010	<0.001
Phenol	<0.001	<0.001	<0.001	15.370	<0.001
Nonpriority organics	0.251	15.870	0.755	11.580	0.149
Total organics	0.255	15.880	0.757	29.972	0.152

Table 7. EC_{50} response in soil (earthworm) soil elutriate and surface water to chemical contaminants in western processing samples.

Test organism	East ditch control	Pond water	005	017	020
			L/L		
Algae	0.450	0.008	0.004	0.249	NE
Daphnia	0.900	0.185	0.033	NE†	NE
Microtox 5 min	NE	0.827	0.412	0.554	NE
15 min	NE	0.213	0.056	0.501	NE
30 min	NE	0.102	0.056	0.434	NE
Lettuce RE	NE	NE	0.614	0.49/1.00‡	NE
Earthworm§	NE	-	>0.50<1.00	>1.00	NE

† NE = No significant toxicity was observed.
‡ 49/100 = 0.49 inhibition in 1.0 soil elutriate.
§ LC_{50} values = concentration at which 0.50 mortality occurs.

content led us to conduct a similar evaluation of heavy metals.

Concentration levels of Cu, Cd, and Zn (Table 8) for East Ditch, pond water and #005 Western Processing samples were sufficiently high to calculate their toxicity potential. These toxicity potential values were obtained by dividing the hardness corrected criteria value for each metal by its corresponding analytical value in each sample. The most toxic predicted level of elutriate was 0.08 L/L for East Ditch, 0.035 for pond water and 0.03 for sample #005. The bioassay EC_{50} values presented in Table 7 show again that algae is the most sensitive test organism to the water soluble constituents in these Western Processing samples. Toxicity to algae ranged from EC_{50} values of 0.004 elutriate in sample #005 to 0.45 of the East Ditch elutriate. No toxicity was observed for any of the test organisms in the #020 elutriate. The earthworm 14-d soil contact test, and the 120 h lettuce seed germination/root elongation test were least sensitive to the contaminants in these samples. Daphnia were most sensitive to #005 and pond water. The 30 min Microtox response was also most sensitive to sample #005 followed in decreasing toxicity by pond water, #017, East Ditch and #020.

The Microtox results suggest different chemical modes of action occurred in the #005 and pond water samples than occurred in sample #017. Samples #005 and pond water increase in Microtox toxicity with increased exposure time while the bioactive components in sample #017 were essentially toxic to the same degree throughout the 5-, 15- and 30-min exposure periods. Increase in toxicity to longer exposure time is characteristic of the response of Microtox to heavy metals (Table 2). Both the toxicity predicted from chemical concentrations and that produced from bioassay, identified #005 and pond water samples as highly toxic. The predicted toxicity for heavy metal appears to be in accord with water quality criteria figures for the #005 and pond water sample, but missed the mark for the East Ditch sample. While the Microtox test is relatively insensitive to cadmium (Table 2) it does respond to Cu and Zn. The combined concentration of Cu and Zn should exceed 1.0 mg/L to produce a 30 min EC_{50} Microtox response. The 1.2 mg/L concentration of these metals in the 0.10 EC_{50} pond water elutriate (Table 8) verifies that these metals were bioactive. Using the same rationale, the toxicity of these metals should produce a 30-min EC_{50} Microtox response of < 0.03 for the #005 elutriate. These bioassay results suggest that toxicity dominated by soluble heavy metals might be accurately predicted from chemistry. However, based on this study, it would appear that toxicity due to priority pollutant organic chemicals most likely cannot be used to accurately predict toxicity. The reason for this is two-fold: (i) the imbalance of priority organics relative to nonpriority organic compounds found in waste site samples; and (ii) the lack of toxicity data and established chemical criteria for organic compounds.

The lack of chemical toxicity data for tens of thousands of important commercial chemicals is reported by a National Research Council Committee (Natural Research Council, 1984) as the major reason that it is impossible to assess their potential hazard to human health. This lack of toxicity data also impedes establishment of environmental and human health criteria for nearly 80% of the chemicals inventoried under the toxic substances control act. Our concern is that criteria established for single chemicals would not solve the definition of hazard assessment at waste sites which contain mixtures of complex chemicals. Even the potential to predict metal toxicity in aqueous samples or elutriates does not contribute significantly to the solution of the problem. According to a hazardous waste

Table 8. Concentration of heavy metals and corresponding estimate of toxicity based on U.S. EPA criteria values for each heavy metal contained in the western processing soil elutriate and surface water samples.

Heavy metal	East ditch			Pondwater			005		
	Criteria value	Analyzed	Estimate of toxicity	Criteria value	Analyzed	Estimate of toxicity	Criteria value	Analyzed	Estimate of toxicity
	mg/L		L/L	mg/L		L/L	mg/L		L/L
Copper	0.002	0.025	0.080	0.45	0.489	0.092	0.009	0.056	0.161
Chromium	0.079	<0.004	NE†	1.80	<0.004	NE	0.400	0.059	NE
Zinc	0.035	0.291	0.174	0.392	11.000	0.036	0.123	42.900	0.003
Nickel	0.150	<0.010	NE	0.870	0.088	NE	0.370	0.116	NE
Cadmium	0.001	<0.003	NE	0.025	0.146	0.171	0.003	0.145	0.021
Hardness of $CaCO_3$		5.6			242			39.5	

† NE = No significant toxic effect is attributed to the heavy metal concentration in the assay sample.

site survey by JRB Associates (1984) heavy metals were the dominant pollutant in soils at 20% of the 225 Superfund sites evaluated. The remaining Superfund sites were contaminated with organics which according to our chemistry and bioassay experience are the most difficult situations for evaluating or predicting toxicity potential at waste sites. Our results indicate that screening level aquatic and terrestrial bioassays can be used to identify potential environmental hazard at waste sites and to prioritize those samples which should receive more rigorous chemical analysis.

SUMMARY AND CONCLUSIONS

Our goal is to develop a realistic bioassay screening protocol. The experimental design and interpretation of bioassay results in the current study have been presented to establish tolerance rankings of each of the test organisms to individual chemicals and major chemical groups to establish response patterns to known elements and compounds. The HMAT test organisms response to complex chemical mixtures contained in the Western Processing samples establishes their ability to ascertain environmental risk associated with hazardous waste sites. Some important conclusions can be drawn from this study.

1. The HMAT protocol for Bioassessment of Hazardous Waste Sites was successfully used to define and rank effects of selected heavy metals, herbicides, and insecticides upon microbes, earthworms, plant seeds, algae, and daphnia.
2. Test organism rank order of sensitivity differs with major toxicant groups assayed.
3. The HMAT test organisms were able to detect the presence of bioactive organic and heavy metal mixtures in Western Processing waste site samples when concentrations did not exceed EPA criteria levels or priority pollutants.
4. Time of exposure effects upon Microtox response may indicate which chemical components contained in complex mixtures are bioactive.
5. Toxicity potential of the Western Processing waste site samples could not be predicted based on priority pollutant organic concentrations. However, calculated toxicity potential associated with metals at two of the five sample sites did agree quite well with toxicity values obtained from direct bioassay.
6. Assuming that bioassays integrate the effects of all toxicants in a sample, it must be concluded that nonpriority pollutants contribute significantly to the toxicity of samples where calculated toxicity potentials for priority pollutant metals and organic constituents do not agree with direct bioassay results.

Additional work along this line needs to be conducted before definitive generalizations can be drawn. The work is on-going at the ERL-Corvallis, OR.

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